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Inhibition of Local Calcergy by Topical Application of Calciphylactic Challengers

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Experiments on rats indicate that, with very few exceptions, the calcification which occurs at subcutaneous sites of treatment with lead acetate, cerium chloride, calcium chloride or potassium permanganate is completely blocked or severely inhibited by simultaneous topical application of calciphylactic challengers but not of non-challengers. Despite the few exceptions (which are thought to depend upon specific chemical interactions between some of the compounds tested) this singular correlation between calciphylactic challenging and anticalcergic potency, is highly significant by the Chi-square-test ($P < 0.001$).

Key words: Calcergy — Calciphylaxis — Calcium — Metals.

Chez le rat, des expériences ont montré que, sauf de rares exceptions, les calcifications produites au site d'injection d'acétate de plomb, de chlorure de cérium, chlorure de calcium ou de permanganate de potassium sont complètement prévenues ou fortement inhibées par l'application locale simultanée de provocateurs calciphylactiques, mais non par des agents non provocateurs. Malgré quelques exceptions (attribuées à des interrelations chimiques spécifiques entre certaines des substances utilisées), cette curieuse corrélation entre l'efficacité calciphylactique provocatrice et le pouvoir anticalcergique est, d'après le test χ^2, hautement significative ($P < 0{,}001$).

Nach subcutaner Injektion von Bleiacetat, Ceriumchlorid, Calciumchlorid oder Kaliumpermanganat tritt an der Injektionsstelle eine starke Verkalkung auf. Bis auf sehr wenige Ausnahmen kann diese durch gleichzeitige lokale Anwendung von calciphylaktischen Provokatoren verhütet oder weitgehend unterdrückt werden. In dieser Beziehung sind nicht provolatorisch wirkende Stoffe inaktiv. Trotz der wenigen Ausnahmen (die wahrscheinlich auf spezifische, chemische Wechselwirkungen zwischen manchen der untersuchten Substanzen beruhen) ist dieser merkwürdige Zusammenhang zwischen calciphylaktisch provozierender und anticalcergischer Aktivität, nach dem χ^2-Test berechnet, statistisch hochsignifikant ($P < 0{,}001$).

Introduction

Calciphylaxis is a phenomenon which can induce selective calcification in various organs; it is brought about by pretreatment with a systemic calcifying compound such as parathyroid hormone or vitamin-D (the "conditioner"), followed after a time interval (the "critical period") by an eliciting agent (the "challenger" — SELYE, 1962). The conditioner is always given systemically and the reaction form is designated as local or systemic, depending upon whether the challenger responsible for the localization of the lesions reaches the target area by direct topical application or through the blood stream (SELYE, 1962; SELYE et al., 1964a).

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Calcergy is a calcinosis, produced without any previous conditioning, by the parenteral administration of certain direct calcifying agents (mostly metallic salts) which are called calcergens (SELYE, 1962; SELYE et al., 1962; GABBIANI et al., 1966). Among these, the salts of lead and of rare earth metals are especially potent. The intravenous administration of several calcergens increases the levels of blood calcium and phosphorus (KUMAGAI and SAKAI, 1966; JOHANNSSON et al., 1968), and so prepares the animals that trauma, or compounds which increase capillary permeability (e.g., histamine liberators, histamine, 5-HT), produce precipitation of calcium salts at the site of their application (SELYE et al., 1962; GABBIANI et al., 1966; SELYE et al., 1964b; SELYE et al., 1964c; GABBIANI and TUCHWEBER, 1965).

Preliminary observations have suggested that topical calcification can be inhibited at the site where a calcergen is applied if simultaneously certain calciphylactic challengers are injected into the same region. On the other hand, agents which do not cause calcification upon subcutaneous injection either in unpretreated or in calciphylactically sensitized rats (that is, compounds which are neither calcergens nor calciphylactic challengers) fail to protect against the topical calcium deposition normally elicited by calcergens (SELYE et al., 1968). This apparently paradoxical fact further emphasizes the essential dissimilarity of calciphylaxis and calcergy.

Here we should like to present the results of investigations in which 72 agents were tested for their ability to inhibit local calcification produced by four calcergens. Through these systematic studies we hoped to determine whether this anticalcifying action in non-sensitized rats is in fact closely related to the calcifying action exhibited by the same compounds in calciphylactically sensitized animals.

Materials and Methods

Female Sprague-Dawley rats with a mean body weight of 100 g (90—110 g) were subdivided into groups of 5—15 animals each and given 200 μg of lead acetate (Pb-ac), cerium chloride ($CeCl_3$) or potassium permanganate ($KMnO_4$), or 2 mg of calcium chloride ($CaCl_2$) in 0.5 ml of distilled water, subcutaneously under the shaved skin of the back. Preliminary experiments had shown that at these dosages all agents tested produce calcified disks having a mean diameter of 11.5—16.5 mm. Each rat received one of these calcergens at one point alone and at a distance of about 3 to 4 cm conjointly with a test substance. Pb-ac, $CeCl_3$ and $CaCl_2$ were administered, the calcergen and the test substances being mixed in 0.5 ml of water, whereas $KMnO_4$ (which caused precipitates with many of the test substances) had to be injected 3 min before the potential inhibitor (each in 0.25 ml of water) at the same spot. In the case of the other calcergens, this sequential injection technique was only used in a few instances when admixture of the potential inhibitor resulted in precipitation (indicated in our tables by asterisks).

The animals were kept on Purina Fox Chow during the experiment and killed with chloroform on the fifth day. The mean diameter of the mineralized disks was measured and the calcific nature of the deposits was subsequently verified histochemically (VON KÓSSA, celestine blue and chloranilic acid tests) on alcohol-formol fixed, paraffin-embedded material as previously described (SELYE, 1962).

The two series of experiments dealt respectively with the inhibition of local calcergy by metals and nonmetallic compounds. The results obtained are listed in our tables together with the calciphylactic challenging potency of the compounds as established by earlier observations (SELYE, 1962). The few compounds not previously tested for this activity have now been assayed under identical conditions.

The metallic compounds used in the *first experimental series* (Table 1) were:

Aluminum chloride ($AlCl_3 \cdot 6\ H_2O$, Fisher Scientific Co., Fair Lawn, N.J., U.S.A.)
Aluminum dextran, "Al-Dex" [an aluminum chelate containing (= 20.4 mg Al/ml), Fisons Pharmaceuticals, Toronto, Canada]
Ammonium chloride (NH_4Cl, Fisher)
Calcium chloride ($CaCl_2$, Fisher)
Cerium chloride ($CeCl_3 \cdot 7\ H_2O$, Fisher)
Cesium chloride (CsCl, Fisher)
Chromium chloride ($CrCl_3 \cdot 6\ H_2O$, Fisher)
Chromium dextran (Cr-Dex, Fisons)
Cupric chloride ($CuCl_2 \cdot 2\ H_2O$, Fisher)
Ferric chloride ($FeCl_3 \cdot 6\ H_2O$, Fisher)
Ferric dextran, "Fe-Dex" [Imferon® (= 50 mg Fe/ml), Fisons]
Ferric dextrin, "Fe-Din" [Astrafer®, a ferric dextrin chelate containing (= 20 mg Fe/ml), Astra, Worcester 6, Mass., U.S.A.]
Ferric oxide, saccharated, "Fe-OS" [Proferrin® (= 20 mg Fe/ml), Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pa., U.S.A.]
Ferric sorbitol, "Fe-SOL" [Jectofer® (= 50 mg Fe/ml), Astra]
Ferrous chloride ($FeCl_2 \cdot 4\ H_2O$, Fisher)
Lanthanum chloride ($LaCl_3 \cdot 7\ H_2O$, Fisher)
Lead acetate, "Pb-ac" [$Pb(C_2H_3O_2)_2 \cdot 3\ H_2O$, Fisher]
Lithium chloride (LiCl, Fisher)
Magnesium chloride ($MgCl_2 \cdot 6\ H_2O$, Fisher)
Manganese chloride ($MnCl_2 \cdot 4\ H_2O$, Fisher)
Mercuric chloride ($HgCl_2$, Fisher)
Nickel chloride ($NiCl_2 \cdot 6\ H_2O$, Fisher)
Potassium chloride (KCl, Fisher)
Potassium iodide (KI, Fisher)
Potassium permanganate ($KMnO_4$, Fisher)
Rhenium heptoxide (Re_2O_7, K & K Laboratories Inc., Plainview, N.Y., U.S.A.)
Rubidium chloride (RbCl, Fisher)
Scandium chloride ($ScCl_3$, K & K)
Selenium dioxide (SeO_2, Matheson Coleman & Bell, Morwood, Cincinnati, U.S.A.)
Silver chloride (AgCl, Fisher)
Sodium chloride (NaCl, Brickman & Co., Montreal, Canada)
Sodium platinic chloride ($Na_2PtCl_6 \cdot 4\ H_2O$, K & K)
Sodium pyrophosphate ($Na_4P_2O_7$, J.T. Baker Chemical Corp., Phillipsburg, N.J., U.S.A.)
Stannous chloride ($SnCl_2 \cdot 2\ H_2O$, Fisher)
Strontium chloride ($SrCl_2 \cdot 6\ H_2O$, Fisher)
Thallium trichloride ($TlCl_3$, Fisher)
Thorium chloride ($ThCl_4$, Fisher)
Thorium dextrin, "Th-Din" [Thorotrast® (= 250 mg Th/ml), Testagar & Co. Inc., Detroit, U.S.A.]
Titanium trichloride ($TiCl_3$, Matheson Coleman & Bell)
Vanadyl sulfate ($VOSO_4 \cdot 2\ H_2O$, Fisher)
Zirconyl chloride ($ZrOCl_2 \cdot 8\ H_2O$, Fisher)

The organic compounds used in the *second experimental series* (Table 2) were:

Actinomycin D (Merck Sharp & Dohme)
Agar I (Agar powder U.S.P., Ac 260 lot No. 460626, Anachemia Chemicals Ltd., Montreal, Canada)
Agar III (Agar-agar U.S.P., lot No. 365, Nutritional Biochemicals Corp. distributed by Brickman Co., Montreal, Canada).
Carrageenin (ordinary impure commercial carrageenan, Marine Colloids Inc., New York, U.S.A.)
Chondroitin sulfate A (Institute for Arteriosclerosis Research, Los Angeles, U.S.A.)
Corn oil (Best Foods Division, Canada Starch Company Ltd., Montreal, Canada)
Dextran (M.W. 75,000, Abbott Laboratories, North Chicago, U.S.A.)

Dextran (M.W. 6,000, Fisons)
Dextrin (British gum, Difco Laboratories, Detroit, U.S.A.)
Egg-white (chicken)
Egg-yolk (chicken)
Endotoxin Coli 08 (Dr. O. Westphal, Max-Planck-Institut f. Immunbiologie, Freiburg, Germany)
Formalin (40% solution of formaldehyde, Fisher)
Gelatin (Fisher)
Glucose (Fisher)
Heparin (Heparin sodium, Nutritional Biochemicals)
India ink ("Higgins" American India ink)
Manucol (Sodium Alginate SS/LD, Alginate Industries Ltd., Girvan, Ayrshire, Scotland)
Ox bile N.F. (Fisher)
Pectin (Sunkist Growers Inc., California, U.S.A.)
Pullulan sulfate (Farbwerke Hoechst A.G., Abteilung, Frankfurt, Germany)
Serum (Fresh rabbit serum)
Sodium caseinate (Nutritional Biochemicals)
Sodium oleate (Fisher)
Sodium palmitate (Delta Chemical Works Inc., New York, U.S.A.)
Sodium salicylate (Fisher)
Tween 20 (Brickman & Co.)
Tween 80 (Brickman & Co.)
Urea (Fisher)

Among the potential inhibitors the calcergens have been tested against other calcergens but not against themselves (lack of data is indicated in the tables by "—"), since earlier work had shown that within the dose-ranges employed here, a rise in the concentration of the calcergen merely increases the resulting calcification.

In the Tables the dosages of metallic chelates, aluminum dextran (Al-Dex), chromium dextran (Cr-Dex), ferric dextran (Fe-Dex) ferric dextrin (Fe-Din), ferric oxysaccharate (Fe-OS), ferric sorbitol (Fe-SOL) and thorium dextrin (Th-Din) are expressed in terms of metal content. Thus the expression "Al-Dex (= 9.7 mg Al)" means that the individual dose of aluminum dextran, contained 9.7 mg of elementary aluminum. Egg-white, egg-yolk, India ink, formaldehyde and rabbit serum are expressed as percentages of the original material.

The tables list only the mean diameters of the calcified disks. It would have been impracticable to discuss the statistical significance of the results obtained by the 72 potential inhibitors in each of the four calcergen-treated series in comparison with each other and with the water-treated controls. However, this proved to be unnecessary for our purpose since, in the control groups treated with the calcergens alone, topical calcification has never failed to occur (either in this or any previous experiment); indeed, the maximum deviation in the diameter of the calcified wheal from the mean rarely exceeded $\pm 20\%$. In view of these circumstances we did not attempt to evaluate borderline inhibitions, considering as significant only total absence of calcification or a reduction to at least 50% of the control value. To facilitate the interpretation of the Tables, the figures reflecting such intense inhibition are printed in bold-face numerals.

In the case of Th-Din (which leaves a white precipitate at the site of injection) and of India ink (which tends to cover the calcified disks), the results of the macroscopic findings were verified not only histochemically (VON KÓSSA, chloranilic acid and celestine-blue techniques) but also (through the kindness of Dr. B. TUCHWEBER) by atomic absorption spectrography.

Results

Since the two tables are self-explanatory, we may limit ourselves here to a few remarks concerning points which necessitate special comment.

The inverse relationship between anticalcergic and calciphylactic challenger potency is especially striking in the series dealing with metals (Table 1). Among the 40 metallic compounds tested, 24 (groups 18—41) were devoid of calci-

Table 1. *Inhibition of local calcergy by various metals*

Group	Potential Inhibitor[a]	Calci-phylactic Challenger	Calcergen			
			Pb-ac 200 μg	$CeCl_3$ 200 μg	$CaCl_2$ 2 mg	$KMnO_4$ 200 μg
			Mean diam. of calcergic wheals (mm)			
1	Water	0	12.5	13.0	16.0	15.5*
2	$AlCl_3$ 500 μg	+	**0**	**0**	**0**	**0***
3	Al-Dex (= 9.7 mg Al)	+	**0**	**0**	**0**	**0***
4	$CrCl_3$ 500 μg	+	**0**	**0**	**0**	**1.0***
5	Cr-Dex (= 9.85 mg Cr)	+	**0**	**0**	**0**	**1.0***
6	$FeCl_2$ 500 μg	+	**1.0**	**0**	**0**	**6.5***
7	$FeCl_3$ 500 μg	+	**2.0**	**0**	**8.0**	**2.5***
8	Fe-Dex (= 6.25 mg Fe)	+	**0**	**0**	**0**	**0***
9	Fe-Din (= 2.5 mg Fe)	+	**0**	**0**	**0**	**3.0***
10	Fe-OS (= 2.5 mg Fe)	+	**0**	**0**	**6.0**	**0***
11	Fe-SOL (= 2.5 mg Fe)	+	**0**	**0**	**0**	**0***
12	Na-Pyrophosphate 2 mg	+	**1.0***	**0**	**3.0**	**0.5***
13	$SnCl_2$ 500 μg	+	**0**	**0**	**1.5**	**3.5***
14	Th-Din (= 31.25 mg Th)	+	**0**	**0**	**Trace**	12.5*
15	$TiCl_3$ 500 μg	+	**0**	**0**	**0**	8.5*
16	$VOSO_4$ 500 μg	+	**0**	**1.0**	**0.5**	12.5*
17	$ZrOCl_2$ 500 μg	+	11.0	**6.0**	10.0	**7.0***
18	$CaCl_2$ 500 μg	CE	12.0	12.5	—	13.5*
19	$CeCl_3$ 500 μg	CE	14.5	—	14.5	13.5*
20	$KMnO_4$ 500 μg	CE	17.0	17.0	20.5	—
21	$LaCl_3$ 500 μg	CE	16.5	16.0	16.5	13.5*
22	Pb-ac 500 μg	CE	—	15.0	15.5	15.5*
23	$ScCl_3$ 500 μg	CE	14.5	15.0	15.0	11.0*
24	$ThCl_4$ 500 μg	CE	14.0	13.5	15.0	13.0*
25	AgCl 500 μg	0	11.5	8.5	15.5	14.5*
26	CsCl 500 μg	0	14.0	10.0	19.0	14.0*
27	$CuCl_2$ 500 μg	0	18.5	18.0	22.0	14.0*
28	$HgCl_2$ 500 μg	0	12.5	14.5	21.5	12.5*
29	KCl 500 μg	0	12.5	9.0	16.0	9.0*
30	KI 500 μg	0	11.0	11.0	15.0	14.0*
31	LiCl 500 μg	0	9.5	9.0	16.0	14.0*
32	$MnCl_2$ 500 μg	0	11.5	10.0	9.5	13.0*
33	$MgCl_2$ 500 μg	0	12.0	10.5	15.0	11.0*
34	Na_2PtCl_6 500 μg	Trace	12.5	11.5	18.0	14.0*
35	NH_4Cl 500 μg	0	12.0	8.0	15.5	14.5*
36	$NiCl_2$ 500 μg	0	12.0	11.0	16.5	13.0*
37	RbCl 500 μg	0	12.0	15.5	17.0	13.5*
38	Re_2O_7 500 μg	0	11.0	13.0	13.0	10.5*
39	SeO_2 500 μg	0	12.5	11.5	18.5	9.0*
40	$SrCl_2$ 500 μg	0	11.0	11.0	18.0	13.0*
41	$TlCl_3$ 500 μg	0	12.5	9.5	15.5	13.5*

[a] The potential inhibitors and calcergens were each administered in 0.25 ml of water, mixed before injection, except in the cases marked "*" were the calcergens had to be injected 3 min before the potential inhibitors, to avoid decomposition.

phylactic challenger potency (including those marked "CE" that, being calcergens themselves, did not even have to be tested in this respect). None of these agents

inhibited the calcification elicited by any of the four test calcergens sufficiently to satisfy the rigorous criteria outlined under "Materials and Methods".

Among the remaining 16 compounds (groups 2—17) with verified calciphylactic challenger potency, 12 inhibited the calcification normally produced by any

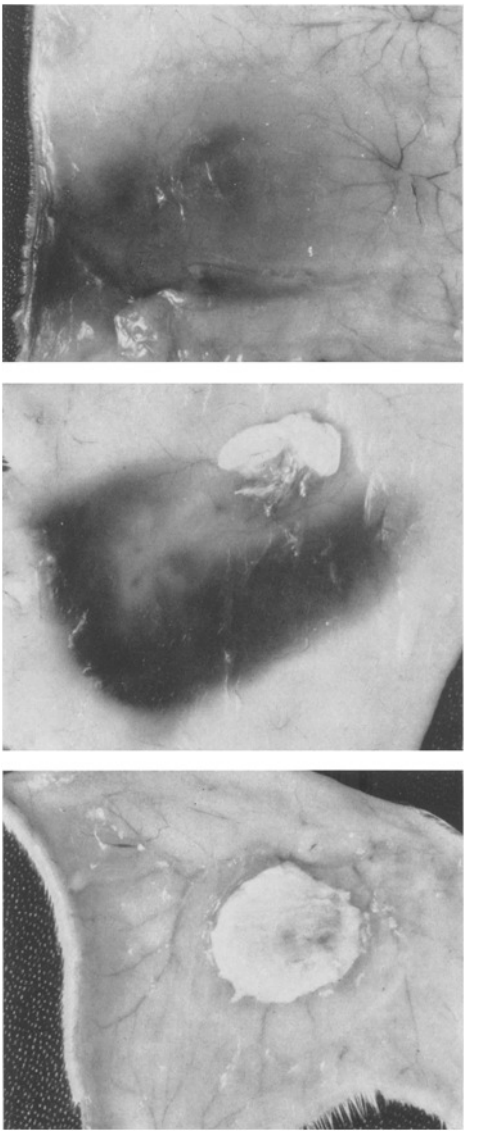

Fig. 1. *Three injection sites on skin lobes viewed from the subcutis. Left:* Calcergy produced by lead acetate. *Middle:* Here, calcergy is only partially inhibited by Fe-Dex because, owing to faulty technique, the lead acetate and Fe-Dex injections did not completely overlap. Prevention of calcification is limited to the dark area covered by the brown Fe-Dex. *Right:* In the other animals of the same series in which the two injections overlapped at all points, calcification was completely prevented

of the four test-calcergens. In most cases this inhibition was complete, but even in the remaining groups it resulted in a decrease of the disk diameter to at least 50% of the control values.

Three additional challengers (Th-Din, $TiCl_3$ and $VOSO_4$) inhibited all calcergens except $KMnO_4$. Here, the lack of protection may well have been due to the fact

Table 2. *Inhibition of local calcergy by various nonmetals*

Group	Potential Inhibitor[a]	Calciphylactic Challenger	Calcergen			
			Pb-ac 200 μg	$CeCl_3$ 200 μg	$CaCl_2$ 2 mg	$KMnO_4$ 200 μg
			Mean diam. of calcergic wheals (mm)			
1	Water	0	11.5	12.0	16.5	14.0*
2	Egg white 25%	+	2.0	0	11.0	13.5
3	Egg-yolk 25%	+	0	0	10.0	6.0
4	Na-caseinate 12.5 mg	+	5.0	0	0	5.0*
5	Na-oleate 2.5 mg	weak	13.0	10.0	5.0	15.0*
6	Na-palmitate 2.5 mg	weak	7.0	6.0	9.0	13.0*
7	Pectin 25 mg	+	5.5	0	0	2.0*
8	Actinomycin 100 μg	0	11.5	12.5	17.0	12.0*
9	Agar III 2.5 mg	0	9.5	10.0	12.5	10.5*
10	Agar I 2.5 mg	0	13.0	8.5	13.5	13.0*
11	Carrageenin (0) 5 mg	0	11.0	1.0	16.0	12.5*
12	Chondroitin SO_4 25 mg	0	12.0	7.5	0	13.0
13	Corn oil	0	12.5*	12.0*	14.0*	13.0*
14	Dextran 25 mg (mol. wt. 6.000)	0	14.0	10.5	17.0	14.0
15	Dextran 15 mg (mol. wt. 75.000)	0	11.0	10.0	15.0	13.0*
16	Dextrin 15 mg	0	11.5	11.0	15.0	13.0*
17	E-coli endotoxin 20 μg	0	13.5	11.0	16.5	14.0*
18	Formaldehyde 0.25%	0	10.5	12.0	16.5	12.0*
19	Formaldehyde 1%	0	9.5	12.5	16.0	12.5*
20	Gelatin 25 mg	0	20.5	9.5	13.5	13.0*
21	Glucose 25 mg	0	10.5	9.5	14.5	13.0*
22	Heparin 0.2 mg	0	10.5	9.0	11.0	12.0*
23	India ink 1%	0	9.0*	10.5	15.0	11.5*
24	India ink 25%	0	0	0	13.5	+++* [b]
25	Manucol 25 mg	0	6.5*	0	0	2.5*
26	Na-salicylate 1 mg	0	12.0	12.5	18.0	13.0*
27	Ox-bile 25 mg	0	11.0	10.0	12.5	13.5*
28	Pullulan 10 mg	0	14.5	2.0	13.0	12.0*
29	Serum (rabbit) 100%	0	16.0	0	2.0	12.5*
30	Tween-20 25 mg	Trace	11.5	10.5	15.0	11.0
31	Tween-80 25 mg	Trace	12.0	11.5	14.5	11.5
32	Urea 25 mg	0	11.0	8.5	15.0	12.5

[a] The potential inhibitors and calcergens were each administered in 0.25 ml of water. The two doses were mixed before injection, except in the cases marked "*" where the calcergens had to be injected 3 min. before the potential inhibitors, to avoid decomposition.

[b] Calcification very pronounced but outlines of disk too diffuse to permit exact measurement.

that (for previously mentioned reasons) $KMnO_4$ — unlike the other calcergens — had to be injected 3 min before the potential inhibitors and even so it may have caused decomposition of the latter.

$ZrOCl_2$ was definitely not as active as most of the other compounds of this group, yet it did inhibit two of the calcergens sufficiently to satisfy our criteria. The calcified disks at the sites treated with the other two calcergens, though not much diminished in diameter, were particularly thin, almost translucent.

The rule of the inverse relationship between calciphylactic challenging and anti calcergic potency is much less evident in the series of 31 organic compounds

(Table 2). Of these only two (Na-caseinate and pectin) inhibited the effect of all four calcergens and — in agreement with expectations — both of these proved to be strong calciphylactic challengers. Egg-white inhibited only two, Na-oleate and Na-palmitate one and egg-yolk three, of the calcergens, despite their calciphylactic challenging potency; yet, all six challengers — even the weak ones — were anticalcergic at least to some extent.

Among the remaining 25 non-challengers (groups 8—32), six (groups 11, 12, 24, 25, 28 and 29) blocked the calcifying effect of some (although in no instance of all) test calcergens.

It is of special interest to us that two normal body constituents, chondroitin sulfate and serum, can prevent the calcification produced by calcium itself. Especially in the case of serum, this may have physiological significance and studies are now under way attempting to identify the responsible serum factor(s). Preliminary observations have shown that whole heparinized rabbit blood is even more effective than serum in that it inhibits not only the $CeCl_3$- and $CaCl_2$- but also the lead acetate-induced calcification although, like serum, it fails to prevent the effect of $KMnO_4$.

Discussion

If we consider all those potential inhibitors as active that inhibit at least one of the four calcergens tested, we may say that all 22 calciphylactic challengers listed in the two tables proved to be active. Conversely, among the 49 compounds which are not calciphylactic challengers (including those which act as calcergens themselves) only six were active in this respect. The statistical significance of this correlation has been checked with the Chi-square-test (CAVALLI-SFORZA, 1961); it was found that the conclusion according to which calciphylactic challengers are calcergic inhibitors whereas non-calciphylactic challengers are not, is highly significant ($P < 0.001$).

Among the metals, the results are quite homogeneous in that none of the compounds devoid of calciphylactic challenger potency inhibited any of the four calcergens tested, whereas all of the calciphylactic challengers proved to be active inhibitors of local calcergy. However, the exceptions require explanation. The fact that Th-Din, $TiCl_3$ and $VOSO_4$ failed to inhibit calcification at the $KMnO_4$-injection site may well have been due to the inactivation of the potential inhibitors by permanganate in contact with tissues. Furthermore, because of its great lability, this particular calcergen had to be injected 3 min before the potential inhibitors, and hence, its calcifying effect upon the surroundings may already have been too firmly established before the blocking action of these particular inhibitors could take effect.

Yet, this kind of argument cannot be invoked to explain those cases where inhibition was limited to the effect of certain calcergens. For example, in Table 1, $ZrOCl_2$ strongly inhibited only the $CeCl_3$ and $KMnO_4$-sites, and in Table 2 only two of the potential inhibitors succeeded in blocking calcifications produced by any of the four calcergens. Of course, it must be remembered that mutually inactivating, specific chemical reactions may take place between certain calcergens and potential inhibitors and that the anticalcifying effect of the latter may also be masked by specific interactions with the surrounding connective tissue.

The main problem is to explain the singular fact that calciphylactic challengers, which induce mineralization in the calciphylactically sensitized animal actually inhibit calcification normally induced by calcergens. One possibility is that the calciphylactic challengers help to solubilize calcium phosphate and, hence, cause a local accumulation of Ca and PO_4 by differential extraction from the blood in the case of hypercalcemia and hyperphosphatemia such as occur after calciphylactic sensitization. In this event, after the challenger is subsequently absorbed, calcium hydroxyapatite would crystalize out of the supersaturated local tissue fluid. On the other hand, when mixed with a calciphylactic challenger (which attracts Ca and PO_4 to the site of its application even during normal calcemia and phosphatemia) both the calcergen itself and any calcium phosphate compound attracted by it, would remain in solution while in contact with the solubilizing challenger. Here this contact may last long enough to permit absorption of all three inorganic constituents (challenger, Ca and PO_4) into the blood before hydroxylapatite precipitation could occur.

There is little to substantiate this view and although it appears to be compatible with most of the observed facts, other possibilities (pH-changes, receptor blockade on collagen, etc.) must also be considered.

Whatever its mechanism, this singular inhibition appears to play a role in several phenomena apart from the blockade of local calcergy. For example, we found that several calciphylactic challengers (Fe-Dex, Cr-Dex, Al-Dex and egg-yolk) given i.v. can protect the rat against otherwise fatal doses of calcergens (e.g., rare-earth metals). Furthermore, the thrombohemorrhagic phenomenon (SELYE, 1966) produced by certain rare-earth metals i.v. and epinephrine or 5-HT s.c. in the rat is likewise inhibited by pretreatment with several of the calciphylactic challengers. Of course, in the blood-clotting mechanism calcium likewise plays an important role and even here the above mentioned hypothesis may apply, but other explanations will also have to be considered.

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